正本

經濟部智慧財產局　函

受文者：國立交通大學（代理人：林火泉　先生）

速別：速件
密等及解密條件：
發文日期：中華民國九十三年一月十二日
發文字號：〈九三〉智專一（一）14005字第〇九三二〇〇三八五五〇號
附件：申請證明文件一份

主旨：檢送第〇九二一三一一九五號專利申請案申請證明文件一份，請　查照。

說明：依九十二年十一月十四日申請書辦理。

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依照分層負責規定授權單位主管決行

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中華民國經濟部智慧財產局

INTELLECTUAL PROPERTY OFFICE
MINISTRY OF ECONOMIC AFFAIRS
REPUBLIC OF CHINA

茲證明所附文件，係本局存檔中原申請案的副本，正確無訛，
其申請資料如下：

This is to certify that annexed is a true copy from the records of this office of the application as originally filed which is identified hereunder:

申　　請　　日：西元 2003 年 11 月 07 日
Application Date

申　請　案　號：092131195
Application No.

申　　請　　人：國立交通大學
Applicant(s)

局　　長
Director General

蔡練生

發文日期：西元 2004 年 1 月 12 日
Issue Date

發文字號：09320038550
Serial No.

發明專利說明書

（本說明書格式、順序及粗體字，請勿任意更動，※記號部分請勿填寫）

※ 申請案號：

※ 申請日期： **※IPC 分類：**

壹、發明名稱：（中文/英文）

生化感測方法及其感測裝置

貳、申 請 人：（共 1 人）

姓名或名稱：（中文/英文）

國立交通大學

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國　籍：(中文/英文)

(均同)中華民國

肆、聲明事項：

☐ 本案係符合專利法第二十條第一項☐第一款但書或☐第二款但書規定之期間，其日期為：　年　月　日。

◎本案申請前已向下列國家（地區）申請專利 ☐ 主張國際優先權：

【格式請依：受理國家（地區）；申請日；申請案號數　順序註記】

1.

2.

3.

4.

5.

☐ 主張國內優先權(專利法第二十五條之一)：

【格式請依：申請日；申請案號數　順序註記】

1.

2.

☐ 主張專利法第二十六條微生物：

☐ 國內微生物 【格式請依：寄存機構；日期；號碼　順序註記】

☐ 國外微生物 【格式請依：寄存國名；機構；日期；號碼　順序註記】

☐ 熟習該項技術者易於獲得，不須寄存。

☑本案技術已公開發表，詳如附件（含部份中譯本）

伍、中文發明摘要：

本發明係一種生化感測方法及其感測裝置，利用此生化感測方法之感測裝置係包括一感光二極體，其可感測特定化合物與特定酵素及顯光劑反應後所產生之光，並能將所感測之光訊號轉換為電流訊號；另有一電流／電壓轉換電路可將該電流訊號轉換為類比電壓訊號；且此類比電壓訊號經由一類比／數位轉換器可再轉換為數位電壓訊號；最後利用一電子裝置可接收此數位電壓訊號並對其加以分析，藉此分析動作以測定該特定化合物之含量多寡。本發明之方法及裝置可提供簡易、大量而即時之醫療檢測，可改善習知檢測技術所使用之光譜分析儀體積過大且價格過高之缺失。

陸、英文發明摘要：

柒、指定代表圖：

(一)、本案代表圖為：第二圖

(二)、本案代表圖之元件代表符號簡單說明：

10	容器	12	感光二極體
14	電流鏡	16	電阻
18	類比／數位轉換器	20	電子裝置
22	電流／電壓轉換電路	24	CMOS 感測晶片
26	電壓供應器		

捌、本案若有化學式時，請揭示最能顯示發明特徵的化學式：

玖、發明說明：

一、【發明所屬之技術領域】

本發明係一種生化感測方法及其感測裝置，特別是一種利用感光二極體感測生化反應所產生之光，以對特定化合物進行定量分析之生化感測方法及其感測裝置。

二、【先前技術】

隨著生物科技及電子科技等現代科學技術的進展，一場全球醫藥革命明顯可期，而在各種醫療檢測的新技術支援下，即時自我診斷已成為可行且重要的趨勢，透過即時的自我健康診斷，一方面可節省就醫時間，另一方面亦可對個人健康進行初步的檢查，因此，研發一種正確性高、攜帶方便、便宜且易於使用的檢測裝置已成為醫藥產業中，最具市場潛力以及最重要的課題。

習用的醫學檢測方法中，光譜分析儀器是一種常用且重要的檢測工具，如分光光度計（Spectrophotometer）等，其係利用光穿透某一物質時，部分波長的光會被該物質吸收之原理，來對該物質進行定性或定量分析，一般利用光譜分析之檢測方式常將待檢測物溶入特定之溶劑中，再將一光源之光入射該溶液，此時一部分的光會被檢測物所形成之溶質所吸收，一部分光會被反射，僅一部分的光會穿透該溶液，而光譜分析儀器便可利用一光感測元件感測此穿透光，並根據其所形成之吸收光譜曲線形狀及強度，比對已知物質之吸收光譜曲線及消光係數，對該物質做定性

及定量分析，諸如血糖檢測、膽固醇檢測、尿酸檢測、膽鹼檢測等都可利用光譜分析儀器進行分析檢測。

然而，傳統的光譜分析儀器係利用光電倍增管（Photomultiplier tube）作為光感測元件，光電倍增管之光陰極在光子作用下會發射電子，這些電子在電場或磁場的加速下會衝擊次極並產生更多的電子，若再將這些電子重覆同樣的加速衝擊過程即可將光電流持續地予以倍增放大，最後，利用一高電位的光陽極即可收集放大後的光電流。而儘管光電倍增管的靈敏度相當高，但由於體積大、價格昂貴且需要高電壓（五百伏特至一千伏特）來加以驅動，故相當程度地限制了所應用之光譜分析儀器的普及性，因此，若一般人想利用光譜分析方式來進行健康檢測，幾乎都得到醫院才能施行。

有鑑於此，本發明提出一種利用感光二極體以進行生化感測之方法及裝置，藉以改善習知技術的缺失。

三、【發明內容】

本發明之主要目的，係提供一種生化感測方法及其感測裝置，該方法及裝置係利用 CMOS 製程所製造之感光元件，來感測生化反應所產生之冷光、螢光、可見光以及紫外光等，藉此減少檢測儀器的成本與體積。

本發明之另一目的，係提供一種生化感測方法及其感測裝

置，藉此提供即時而大量之檢測。

本發明之又一目的，係提供一種生化感測方法及其感測裝置，藉此將生化醫學檢測簡化為一般人均可自行操作的程序。

根據本發明之生化感測方法，首先將一待測溶液中之特定化合物，如血糖、膽固醇、尿酸等，與第一酵素反應並產生過氧化氫，接著再利用顯光劑及第二酵素與過氧化氫反應以產生化學發光，此時利用一半導體製程所製造之感光元件感測該光，並將光訊號轉換為電流訊號，接著利用一電流／電壓轉換電路將電流訊號轉換為類比電壓訊號，再利用一類比／數位轉換器將類比電壓訊號轉換為數位電壓訊號，最後藉由一電子裝置對此數位電壓訊號進行分析，以獲得於待測溶液中特定化合物之濃度。

又根據本發明之生化感測裝置，其係包括一承載體，此承載體承載有特定化合物、顯光劑及第一、第二酵素混合而成之冷光檢測試劑，此冷光檢測試劑所產生之化學發光會被一半導體製程所製造之感光元件加以感測，此感光元件更會將所感測之光轉換為電流訊號，另有一電流／電壓轉換電路，其可將該電流訊號轉換為類比電壓訊號，而此類比電壓訊號經由一類比／數位轉換器可轉換為數位電壓訊號，最後尚有一電子裝置接收此數位電壓訊號，並對其進行分析、儲存或傳送之動作。

底下藉由具體實施例的說明，並參照所附之圖式，當更容易瞭解本發明之目的、技術內容、特點及其所達成之功效。

四、【實施方式】

本發明係一種生化感測方法及其感測裝置，利用此方法及感測裝置，可減少檢測儀器的成本與體積，並可提供一般人進行即時自我健康檢測，以減少就醫時間及成本。

參照第一圖，其係本發明之生化感測方法流程示意圖，如圖所示，首先於步驟S10中將含有特定化合物之待測溶液與含有第一酵素之溶液混合在一起，並使此混合溶液產生化學反應而生成過氧化氫，該特定化合物可為血糖（Glucose）、膽固醇（Cholesterol）、尿酸（Uric acid）、乳酸鹽（Lactate）、磷脂（Phospholipids）、及三酸甘油酯（Triglycerides）等人體化合物，而第一酵素則係配合特定化合物之種類而適當選擇，舉例而言，該第一酵素之種類可為葡萄糖氧化酵素（Glucose oxidase）、膽固醇氧化酵素（Cholesterol oxidase）、尿酵素（Uricase）、乳酸氧化酵素（Lactate oxidase）、磷脂酵素（Phospholipase）、及脂肪分解酵素（Lipase）等等；執行完步驟S10後，接著於步驟S12中，利用顯光劑及過氧化酵素（Peroxidase）與步驟S10所生成之過氧化氫反應產生化學發光，藉此化學發光反應可產生冷光、螢光、可見光或紫外光等，而步驟S12中所使用之顯光劑係選自光敏靈（Luminol）、二甲基引朵（2-methyl Indole）、異氨基苯二甲酰肼（Isoluminol）、二氧環烷（dioxetane）、吖啶翁

酯（Acridinium ester）、亮光素（lucigenin）、金剛烷二氧丁環磷酸鹽（AMPPD）及 CDP-Star、CSPD 等化學冷光試劑；待執行完步驟 S12 後即執行步驟 S14，亦即利用至少一半導體製程所製造之感光元件，如感光二極體（Photodiode）等，來感測步驟 S12 所產生之光，該感光二極體並可將所感測之光訊號轉換為電流訊號；接著，於步驟 S16 中，利用一電流／電壓轉換電路將前述電流訊號轉換為類比電壓訊號，且此電流／電壓轉換電路可利用電流鏡之設計將電流訊號放大，並可利用電阻或電容將電流訊號轉換為類比電壓訊號；接著，執行完步驟 S16 後即執行步驟 S18，即利用一類比／數位轉換器將類比電壓訊號再轉換成數位電壓訊號；最後於步驟 S20 中，利用可攜式顯示器、個人電腦或工作站等電子裝置將該數位電壓訊號加以分析、儲存或傳送，並藉此獲得該待測溶液中，該特定化合物含量多寡等資訊。

參照第二圖，其係本發明生化感測裝置之方塊示意圖，如圖所示，一容器 10 中盛有待測定之生化冷光檢測試劑，此試劑中含有特定化合物、第一酵素、顯光劑及過氧化酵素，該特定化合物會與第一酵素反應產生過氧化氫，而此過氧化氫會再與顯光劑及過氧化酵素反應產生冷光、螢光、可見光或紫外光等，而此化學發光所產生之光會再由一感光二極體 12 加以感測，且此感光二極體 12 會將所感測之光訊號轉換為電流訊號，然而，為避免此電流訊號過於微弱而難以辨識處理，此電流訊號會再經電流鏡 14 加以

放大，且放大後的電流訊號會再經一電阻 16 轉換為類比電壓訊號以利於進行訊號處理，又另有一類比／數位轉換器 18 可將此類比電壓訊號轉換為數位電壓訊號，且此數位電壓訊號會被一電子裝置 20 所接收，並由其進行分析、儲存、或傳送等動作，藉此可獲得於該試劑中，該特定化合物濃度大小等資訊。

承上所述並參照第二圖，其中該容器 10 亦可為石英管、試片等承載體，而其所盛之特定化合物可為血糖、膽固醇、尿酸、乳酸鹽、磷脂、及三酸甘油酯等人體化合物，而該第一酵素係配合特定化合物之種類而加以適當選擇，另該顯光劑可選自光敏靈、二甲基引朵、異氨基苯二甲酰肼、二氧環烷、吖啶翁酯、亮光素、金剛烷二氧丁環磷酸鹽及 CDP-Star、CSPD 等化學冷光試劑，此外，該電流鏡 14 及電阻 16 可整合於一電流／電壓轉換電路 22 內，且電阻 16 可由電容加以取代，藉此亦可將電流訊號轉換成類比電壓訊號以輸出至類比／數位轉換器，另感光二極體 12、電流鏡 14 及電阻 16 更可整合於一 CMOS 感測晶片 24 上，使該 CMOS 感測晶片 24 同時具有感測化學發光及將光訊號轉換為類比電壓訊號以輸出之功能，如第三圖所示。

參照第四圖，其係本發明之生化感測裝置示意圖，圖中所示之 CMOS 感測晶片 24 係包含前述感光二極體及電流／電壓轉換電路，一容器 10 則置放於此 CMOS 感測晶片 24 上，此容器 10 中裝有生化冷光檢測試劑，此試劑發生之化學發光反應所產生之冷

光、螢光、可見光或紫外光可由該CMOS感測晶片24加以感測，並由其將光訊號轉換為類比電壓訊號以輸出至一類比／數位轉換器18，而此類比／數位轉換器18會再將類比電壓訊號轉換為數位電壓訊號以輸出至一電子裝置20，並藉由此電子裝置20對該數位電壓訊號加以分析、儲存或傳送，此外，尚有一電壓供應器26可供應CMOS感測晶片24穩定之電壓源。

另外，本發明亦針對葡萄糖做定量分析以驗証本發明之可行性，首先，將葡萄糖與葡萄糖氧化酵素反應以生成過氧化氫，其化學反應式為：葡萄糖＋O_2＋H_2O＋葡萄糖氧化酵素→葡萄糖酸＋H_2O_2；接著再將過氧化氫（H_2O_2）與山葵根過氧化酵素（Horseradish Peroxidase）反應產生化學發光，其化學反應式為：$2H_2O_2$＋光敏靈＋山葵根過氧化酵素→3-氨基鄰苯二甲酸＋N_2＋波長425奈米之光；最後再藉由本發明之生化感測裝置感測化學發光反應所產生之光，並藉以測定葡萄糖之量，而第五圖至第七圖依序為利用本發明所測得之山葵根過氧化酵素活性檢量線、過氧化氫檢量線及葡萄糖檢量線，由第五圖可知，當山葵根過氧化酵素份量在1單位以下時，山葵根過氧化酵素的活性與本發明之生化感測裝置所檢測之電壓成正比，且山葵根過氧化酵素一單位定義為：於pH值6.0且溫度20℃下，20秒中可將焦培酚（Pyrogallol）催化出1mg之Purpurogallin；第六圖則顯示出過氧化氫濃度與檢測電壓之關係；而由第七圖之葡萄糖檢量線可知，當葡萄糖濃度在5mM

以下時，若葡萄糖濃度產生微小變化，檢測電壓會隨之產生明顯之變化，故本發明確可利用此葡萄糖檢量線對葡萄糖做精確之定量分析。

由上所述，本發明係利用成熟CMOS製程所製造之CMOS感測晶片，感測化學發光反應所產生之光，並再利用類比／數位轉換器及可攜式顯示器等電子裝置，處理CMOS感測晶片所感測之訊號，而由於CMOS感測晶片具有體積小、低成本及能夠大量生產製造等優點，且類比／數位轉換器亦可整合於CMOS感測晶片中或整合於電子裝置裡，故本發明能夠提供低成本、準確、即時且方便之檢測，讓使用者無需就醫即可進行自我健康檢查，並能達到節省就醫時間、降低醫療成本及隨時檢測身體健康狀況之功效。

惟以上所述之實施例僅為本發明之較佳實施例而已，並非用以限定本發明實施之範圍。故凡依本發明申請專利範圍所述之形狀、構造、特徵及精神所為之均等變化與修飾，均應包括於本創作之申請專利範圍內。

五、【圖式簡單說明】

圖式說明：

第一圖為本發明之生化感測方法流程示意圖。

第二圖為本發明之生化感測裝置方塊示意圖。

第三圖為本發明CMOS感測晶片電路示意圖。

第四圖為本發明之生化感測裝置示意圖。

第五圖為利用本發明所量得之山葵根過氧化酵素檢量線示意圖。

第六圖為利用本發明所量得之過氧化氫檢量線示意圖。

第七圖為利用本發明所量得之葡萄糖檢量線示意圖。

圖號說明：

10	容器	12	感光二極體
14	電流鏡	16	電阻
18	類比／數位轉換器	20	電子裝置
22	電流／電壓轉換電路	24	CMOS 感測晶片
26	電壓供應器		

拾、申請專利範圍：

1、一種生化感測方法，該生化感測方法之步驟包括：

將一含有特定化合物之待測溶液加入一溶液中，該溶液中含有一第一酵素，該第一酵素能與該特定化合物產生化學反應，並生成過氧化氫；

利用一顯光劑及一第二酵素與該過氧化氫形成化學發光反應；

利用至少一半導體製程所製造之感光元件感測該化學發光反應所產生之光，且該感光元件會將所感測之光訊號轉化為電流訊號；

將該電流訊號轉換為電壓訊號；以及

根據該電壓訊號，對該特定化合物進行定量分析，藉以測定於該溶液中，該特定化合物之含量多寡。

2、如申請專利範圍第 1 項所述之生化感測方法，其中該特定化合物係選自血糖、膽固醇、尿酸、乳酸鹽、磷脂、及三酸甘油酯等人體化合物。

3、如申請專利範圍第 1 項所述之生化感測方法，其中該顯光劑可選自光敏靈、二甲基引朵、異氨基苯二甲酰肼、二氧環烷、吖啶翁酯、亮光素、金剛烷二氧丁環磷酸鹽、CDP-Star 及 CSPD 等化學冷光試劑。

4、如申請專利範圍第 1 項所述之生化感測方法，其中該第一酵素係根據該特定化合物之種類而適當選擇。

5、如申請專利範圍第 1 項所述之生化感測方法，其中該第二酵素

係過氧化酵素。

6、如申請專利範圍第 1 項所述之生化感測方法，其中該感光元件可為一感光二極體。

7、如申請專利範圍第 1 項所述之生化感測方法，其中該感光元件係設計用以感測冷光、螢光、可見光、紫外光及上述四者之任意組合。

8、如申請專利範圍第 1 項所述之生化感測方法，其中該電流訊號轉換為該電壓訊號之步驟係直接將該電流訊號轉換為類比電壓訊號。

9、如申請專利範圍第 1 項所述之生化感測方法，其中該電流訊號轉換為該電壓訊號之步驟係包括將該電流訊號轉換為類比電壓訊號，以及再將該類比電壓訊號轉換為數位電壓訊號。

10、一種生化感測裝置，該生化感測裝置包括：

一承載體，其上承載有一試劑，該試劑含有一特定化合物、一第一酵素、一第二酵素及一顯光劑，該特定化合物會與該第一酵素反應產生過氧化氫，該過氧化氫會與該第二酵素及該顯光劑產生化學發光反應；

一感光元件，其係利用半導體製程所加以製造，該感光元件可感測該化學發光反應所產生之光，且會將所感測之光訊號轉化為電流訊號；

一電流／電壓轉換電路，其可將該電流訊號轉換為電壓訊

號；以及

一電子裝置，其可接收並處理該電壓訊號，藉此可對該特定化合物進行後續之定量分析動作。

11、如申請專利範圍第 10 項所述之生化感測裝置，其中該特定化合物係選自血糖、膽固醇、尿酸、乳酸鹽、磷脂、及三酸甘油酯等人體化合物。

12、如申請專利範圍第 10 項所述之生化感測裝置，其中該顯光劑可選自光敏靈、二甲基引朵、異氨基苯二甲酰肼、二氧環烷、吖啶翁酯、亮光素、金剛烷二氧丁環磷酸鹽、CDP-Star 及 CSPD 等化學冷光試劑。

13、如申請專利範圍第 10 項所述之生化感測裝置，其中該第一酵素係根據該特定化合物之種類而適當選擇。

14、如申請專利範圍第 10 項所述之生化感測裝置，其中該第二酵素係過氧化酵素。

15、如申請專利範圍第 10 項所述之生化感測裝置，其中該感光元件可為一感光二極體。

16、如申請專利範圍第 10 項所述之生化感測裝置，其中該感光元件係設計用以感測冷光、螢光、可見光、紫外光及上述四者之任意組合。

17、如申請專利範圍第 10 項所述之生化感測裝置，其中該電流／電壓轉換電路可包括至少一電流鏡，其可將該電流訊號放大。

18、如申請專利範圍第 10 項所述之生化感測方法，其中該電流／電壓轉換電路可包括至少一電阻，其可將該電流訊號轉換成類比電壓訊號。

19、如申請專利範圍第 10 項所述之生化感測裝置，其中該電流／電壓轉換電路可包括至少一電容，其可將該電流訊號轉換成類比電壓訊號。

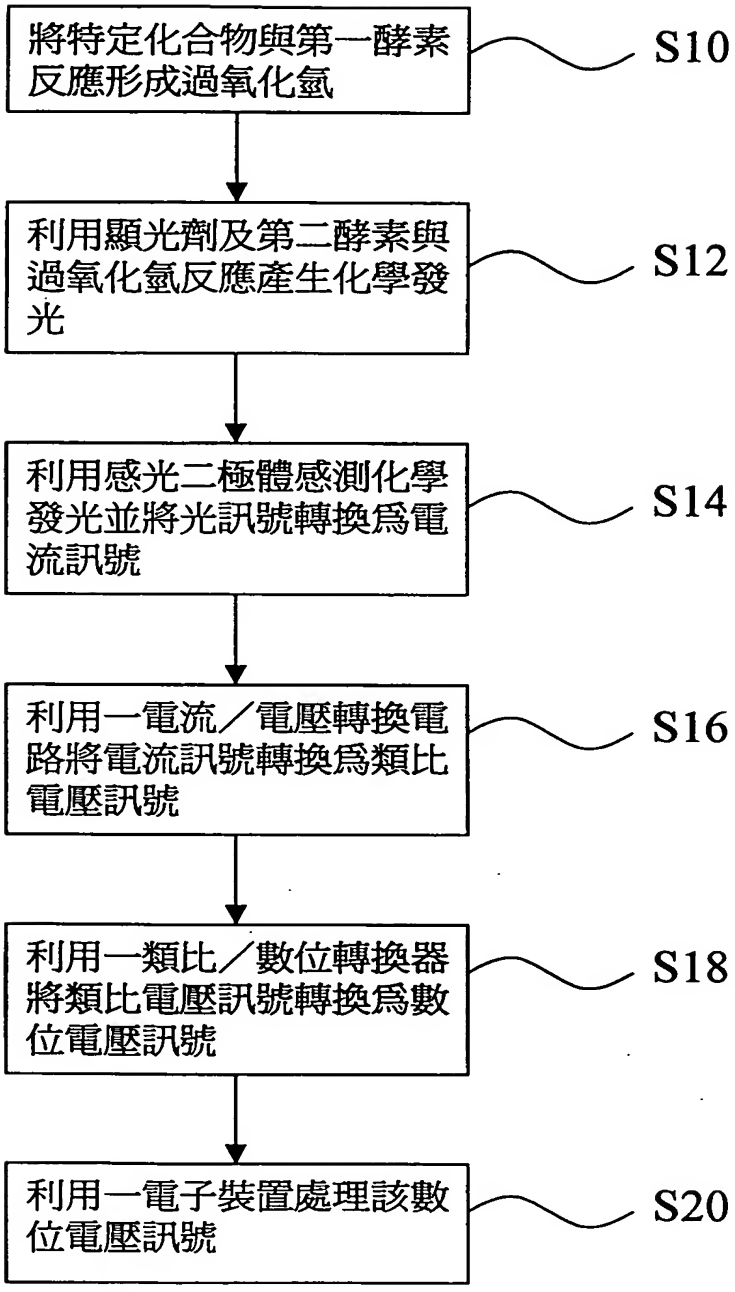
20、 如申請專利範圍第 18 項所述之生化感測裝置，其中該電流／電壓轉換電路可包括一類比／數位轉換器，其可將該類比電壓訊號轉換為該數位電壓訊號。

21、 如申請專利範圍第 19 項所述之生化感測裝置，其中該電流／電壓轉換電路可包括一類比／數位轉換器，其可將該類比電壓訊號轉換為該數位電壓訊號。

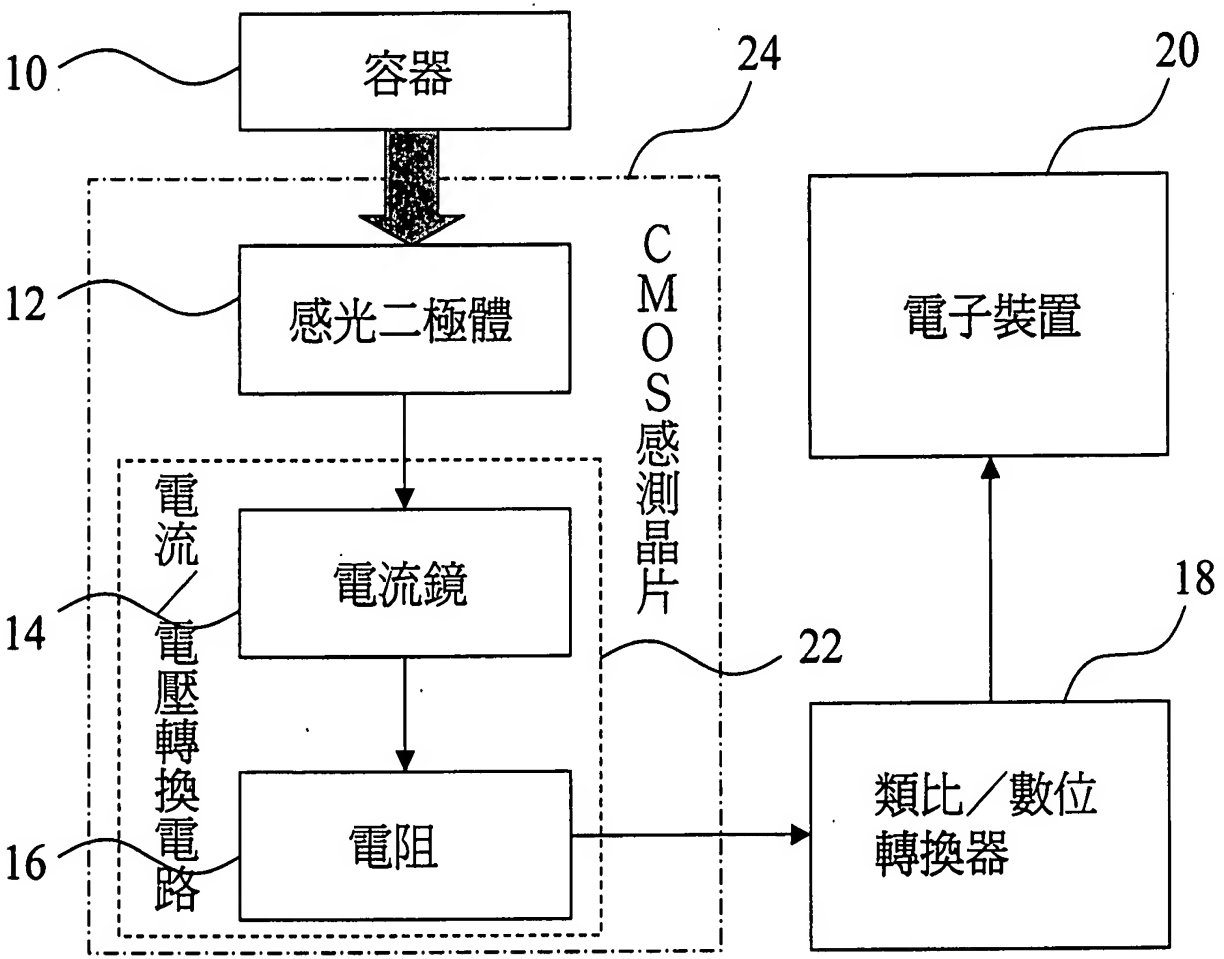
22、如申請專利範圍第 18 項所述之生化感測裝置，其中該電子裝置可包括一類比／數位轉換器，其可接受該類比電壓訊號，並將其轉換為數位電壓訊號。

23、如申請專利範圍第 19 項所述之生化感測裝置，其中該電子裝置可包括一類比／數位轉換器，其可接受該類比電壓訊號，並將其轉換為數位電壓訊號。

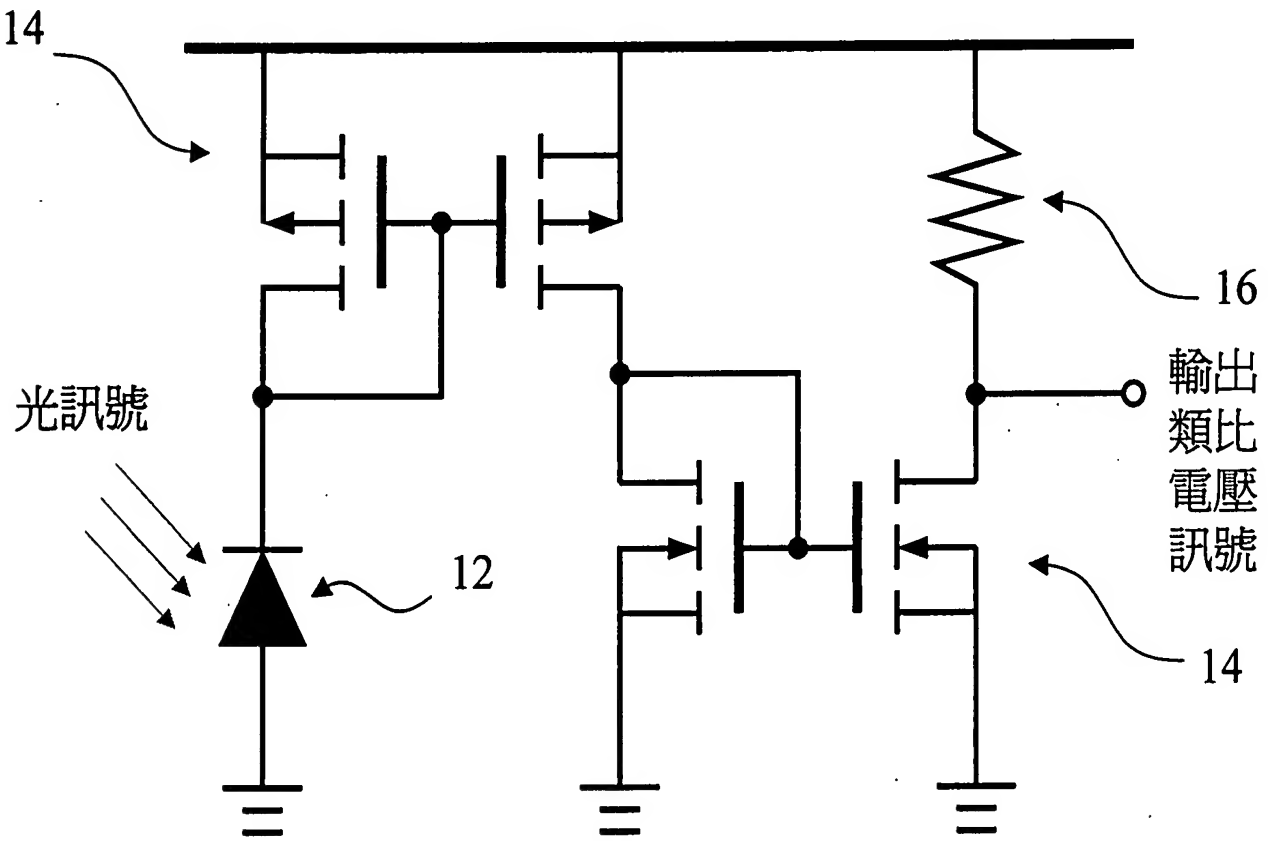
24、如申請專利範圍第 10 項所述之生化感測裝置，其中該電子裝置對該電壓訊號之處理方式係選自傳送、儲存、分析及前述三者任意組合。


將特定化合物與第一酵素反應形成過氧化氫
S10
利用顯光劑及第二酵素與過氧化氫反應產生化學發光
S12
利用感光二極體感測化學發光並將光訊號轉換爲電流訊號
S14
利用一電流／電壓轉換電路將電流訊號轉換爲類比電壓訊號
S16
利用一類比／數位轉換器將類比電壓訊號轉換爲數位電壓訊號
S18
利用一電子裝置處理該數位電壓訊號
S20

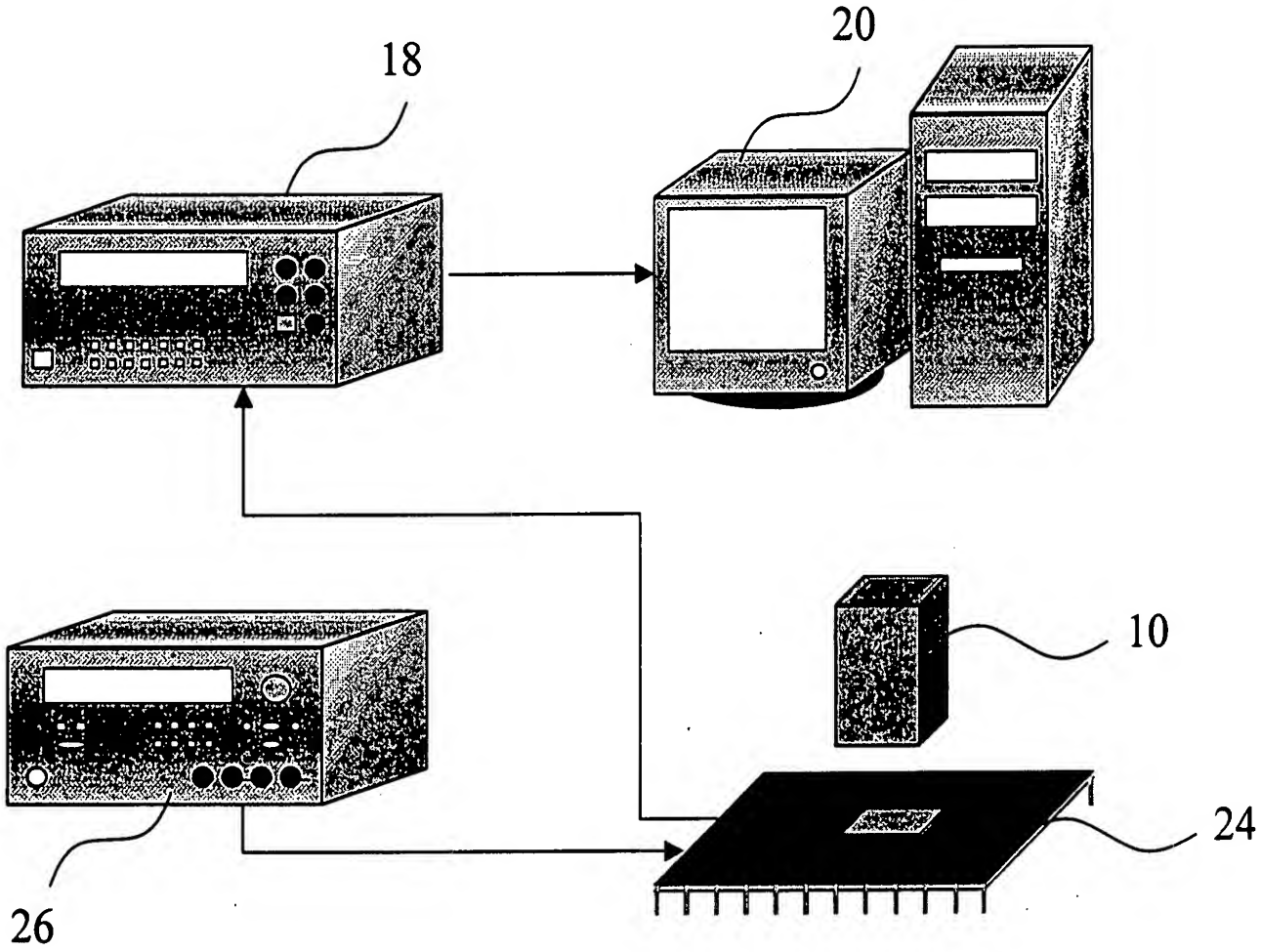
第一圖


10
容器
24
20
CMOS感測晶片
12
感光二極體
電子裝置
電流
電壓轉換電路
14
電流鏡
18
22
16
電阻
類比／數位
轉換器

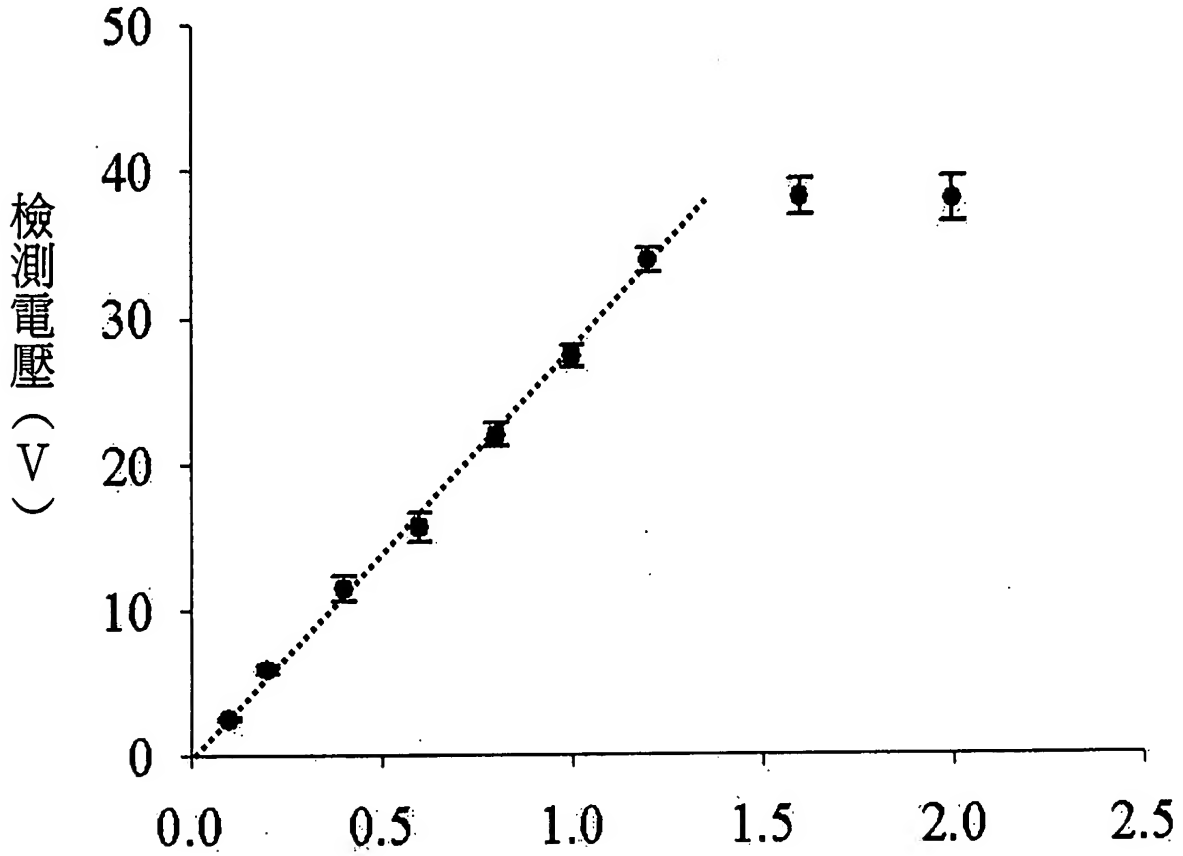
第二圖



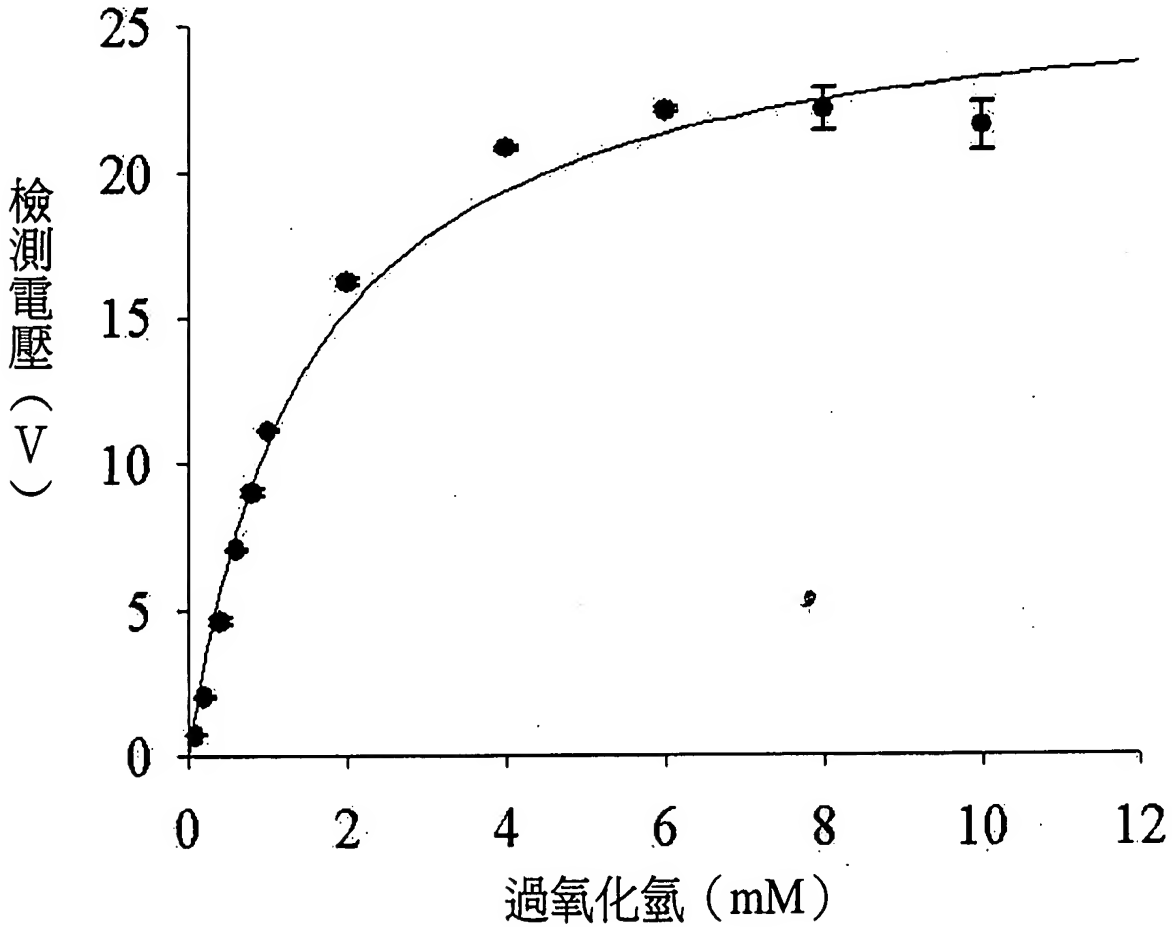
第三圖



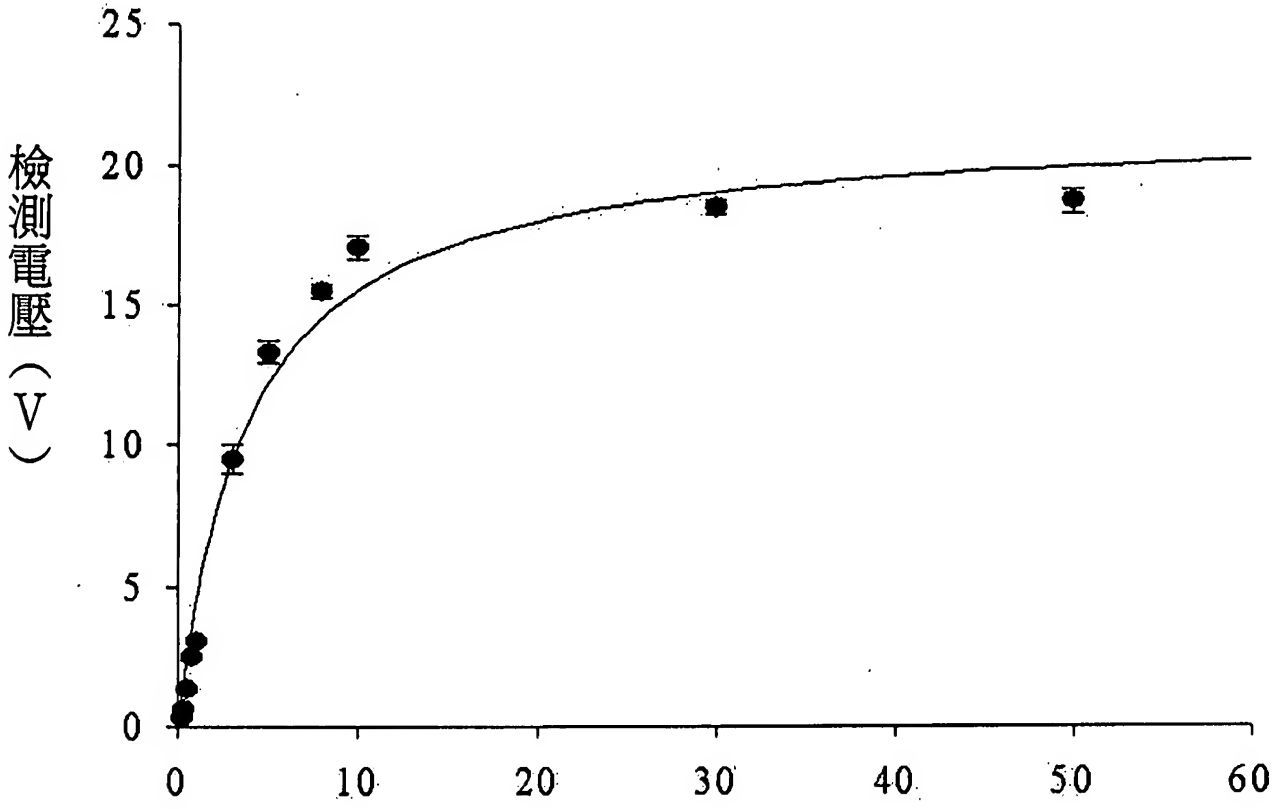
第四圖



第五圖



第六圖



第七圖

CMOS Chip as Luminescent Sensor for Biochemical Reactions

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Abstract—**We describe a novel biochemical sensing method and its potential new biosensing applications. A light-sensitive complementary metal oxide semiconductor (CMOS) chip prepared through a standard 0.5-μm CMOS process was developed for measuring biochemical reactions. A light producing enzymatic reaction catalyzed by horseradish peroxidase (HRP) was designed as a platform reaction to determine the concentration of hydrogen peroxide (H_2O_2) by the CMOS chip with a standard semiconductor parameter analyzer (HP4145). The kinetics of enzymatic reaction were determined and compared with a standard and sophisticated fluorometer (Hitachi F-4500) in a biochemical laboratory. Similar results were obtained by both instruments. Using glucose oxidase as an example, we further demonstrated that the HRP platform can be used to determine other H_2O_2 producing reactions with the CMOS system. The result points to an important application of the CMOS chip in biological measurements and in diagnosis of various health factors.**

I. Introduction

RAPID advancement in modern biology has produced opportunities and needs in clinical diagnostics and many other biorelated measurements. To develop a high-throughput instrument with small size, low cost, ease of use, and high accuracy is becoming more and more important. Development of a suitable sensor for a variety of biochemical reactions is the key for this progress [1]. The small size of semiconductor sensors not only contributes to their potentially low cost, but also allows them to be integrated with microelectronic circuit, creating the so called integrated sensors, further enhancing their performance [2].

Optical instruments, such as UV-vis absorption spectrophotometer, fluorescence meters, and luminescence meters are routinely used to measure biochemical reactions, enabling bio- and chemiluminescence to become powerful tools for assaying a variety of biologically important molecules [3]. The photomultiplier tube (PMT) is the most common light sensor used in these spectrophotometers. PMT is an effective and sensitive light sensor, wherein one photon can induce approximately 10^6 electrons in the photomultiplier tube. However, the need for high voltage (about 500 to 1000 V), the size, and the price of the PMT limit its application in a variety other fields such as personalized diagnosis kits.

The complementary metal oxide demiconductor (CMOS) process is the most commonly used procedure in semiconductor industry. A photodiode is basically a p-n junction operated under reverse bias. Free electron-hole pairs will be generated in photodiode when photodiode is illuminated by the photon, which contains energy higher than the band gap of photodiode [4]. CMOS photodiodes act as semiconductor light sensor with the advantages of low price, small size, and low power consumption as compares to that of PMT. These features make CMOS photodiodes easy to be a personalized healthy care instrument, and a high-throughput sensor. Recently, a CMOS photodiode has been used to monitor *Pseudomonas fluorescens 5RL* bacterial cells [5]. However, to our knowledge, there have been no reports in which a biochemical reaction has been coupled to CMOS photodiodes. We demonstrated a reaction (1) that can be used as the platform light emitting reaction to permit enzymatic activity to be observed

H_2O_2 detection (Luminol/HRP/H_2O_2) system

$$2H_2O_2 + \text{luminol} \xrightarrow{\text{Peroxidase}} 3-\text{aminophthalate} + N_2 + \text{Light}(425\ \text{nm})$$

(1)

which shows the light emitting reaction catalyzed by horseradish peroxidase (HRP). Since many enzymes produce H_2O_2 (Table I and [6]), the HRP-luminol-H_2O_2 system should be a good platform reaction to couple and detect many other enzymatic reactions by luminescence. In a CMOS photodiode system, the chemiluminescence generated from the biochemical reaction produces current flow that corresponds to the rate of enzymatic-catalyzed reaction. By using this luminescent-coupled enzyme assay, we can easily translate the concentration of

Manuscript received May 12, 2002; revised February 14, 2003. This work was supported in part by a grant from Hong Wei Biotech and in part by the National Science Council (NSC), Taiwan, R.O.C., under Grant 90-2311-B-009-002. The associate editor coordinating the review of this paper and approving it for publication was Dr. Gerard L. Coté.

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Digital Object Identifier 10.1109/JSEN.2003.814653

1530-437X/03$17.00 © 2003 IEEE

TABLE I
IMPORTANT BIOCHEMICALS THAT CAN BE DETERMINED BY HRP-luminol-H_2O_2 SYSTEM

Target	Enzyme coupled	H_2O_2 producing reactions
Glucose	Glucose oxidase	$Glucose + O_2 + 2H_2O \xrightarrow{Glucose\ Oxidase} Gluconic\ acid + 2H_2O_2$
Uric acid	Uricase	$Uric\ acid + O_2 + 2H_2O \xrightarrow{Uricase} allantoin + CO_2 + 2H_2O_2$
Cholesterol	Cholesterol oxidase	$Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} cholesten\text{-}3\text{-}one + H_2O_2$
Lactate	Lactate oxidase	$L\text{-}Lactate + O_2 \xrightarrow{Lactate\ oxidase} pyruvate + H_2O_2$
Phospholipids	Phospholipase/ Choline oxidase	$Phospholipids + H_2O \xrightarrow{Phospholipase} Fatty\ acids + Choline$ $Choline + H_2O + 2O_2 \xrightarrow{Choline\ oxidase} betaine + 2H_2O_2$
Triglycerides / Lipase	Lipase/ Glycerol oxidase	$Triglycerides + 3H_2O \xrightarrow{Lipase} Fatty\ acids + glycerol$ $Glycerol + O_2 \xrightarrow{Glycerol\ oxidase} glyceraldehyde + H_2O_2$

specific compound to luminescence. In this paper, we choose glucose/glucose oxidase system [as seen in (2), at the bottom f the page] to demonstrate that a CMOS chip can be used to measure this frequently used clinical assay.

II. EXPERIMENTAL

A. Materials

D(+)−Glucose, horseradish peroxidase, luminol, and Bis-Tris propane were purchased from Sigma. H_2O_2 (30%, W/W) and H_2KPO_4 were purchased from Riedel-deHaën. Glucose oxidase (GOD, from *Aspergillus niger*) was purchased from Fluka. Tris-HCl buffer is purchased from Pharmacia Biotech. K_2HPO_4 was obtained from J. T. Baker.

B. Photomultiplier Tube (PMT) Detection System

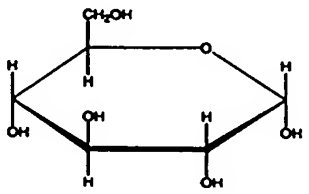
HITACHI F-4500 fluorescence spectrometer was used as control sensor for spectrophotometry in this study. To btain comparable luminescent data between the fluorescence spectrometer and the CMOS photodiode, the instrumental parameters were set as follows: PMT voltage, 700 V; emission wavelength, 425 nm; and emission slit, 1 or 2.5 nm. The choice of emission slit was dependent on the strength of the luminescence observed. The emission slit at 2.5 nm observes 8 to 10 times luminescence compared with that of the 1-nm slit used.

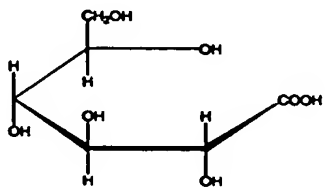
C. CMOS/HP4145 Detection System

As shown in Fig. 1, the CMOS chip used for this study was manufactured by CMOS 0.5-μm standard process. The CMOS photodiode [Fig. 1(a)] and layout [Fig. 1(b) and (c)] diagram of N^+/P well photodiodes array has 140 × 240 photodiodes with single pixel size 7.5 μm × 7.5 μm. The photodiode chip has no color filter, so it absorbs the full luminescence spectrum of chemical reaction (from 400 to 800 nm). The chemiluminescence-generated current of CMOS photodiodes was in pico ampere level, and a sensitive semiconductor parameter analyzer was needed to determine this small difference.

We used HP 4145 semiconductor parameter analyzer with a chip-fixing box HP 16058A test fixture to collect and analyze the chemiluminescence-generated current of N^+/P well photodiodes. The structure diagram of the chemiluminescence-generated current monitoring system was shown in Fig. 2. A 3.3-V

H_2O_2generating (Glucose/Glucose Oxidase) system $\quad Glucose + O_2 + H_2O \xrightarrow{GlucoseOxidase} Gluconic\ acid + H_2O_2$ (2)





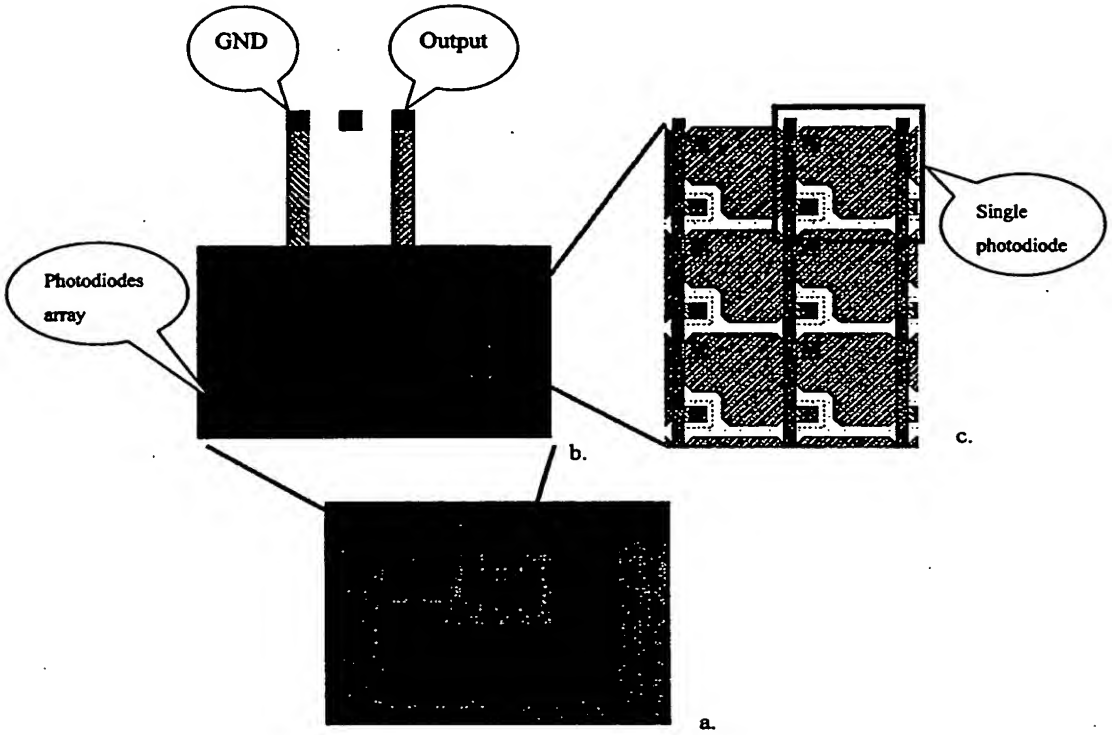


Fig. 1. CMOS photodiode as light sensor. (a) The CMOS photodiode manufactured by 0.5-μm standard process after packaging. (b) Layout of CMOS photodiodes. The photodiode array is 140*240. (c) The size of a single photodiode is (7.5 μm)^2.

reverse bias was given to CMOS photodiodes chip by HP 4145. The reaction condition was the same as that used by PMT detection system (Hitachi F4500) described later except that 0.2 unit of HRP is used.

D. Preparation and Use of Enzymes

HRP powder (1 mg or 80 units) was dissolved in 1-ml Tris-HCl buffer (0.1 M at pH 8.6). The powder of Glucose oxidase (20 mg or 3000 units) was dissolved in 1 ml phosphate buffer (0.2 M at pH7.0). The stock enzyme solution was stored at −80 °C. The HRP and GOD solutions were melted in an ice bath just before use and were diluted with specified buffer. An aliquot amount of enzyme was added into the cuvette followed by the injection of all other necessary reagents and sample (as described later for reaction conditions) in order to make sure that all compounds were well mixed in cuvette without extra shaking and that the data can be collected in a short period of time. One unit of HRP means 1.0 mg of purpurogallin is formed from pyrogallol in 20 s at pH 6.0 at 20 °C as described by Sigma. One unit of GOD will oxidize 1-μmol glucose per minute at pH 7.0 and 25 °C as determined by Fluka.

E. Reaction Condition of HRP-luminol-H_2O_2 System

The optimal condition for HRP-luminol-H_2O_2 system was determined, as shown in Figs. 3 and 4. Unless otherwise specified, the standard condition involved HRP-luminol-H_2O_2 system included luminol (1.5 mM,), H_2O_2 (3 mM), and Tris-HCl (100 mM at pH 8.6) and HRP (0.008 units) at 25 °C.

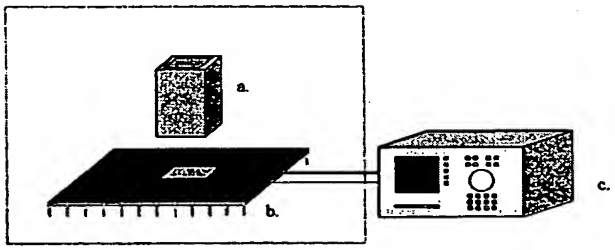


Fig. 2. Schematic diagram of the CMOS photodiodes/HP 4145 detection system. (a) Quartz cuvette (the reaction chamber) on the top of CMOS photodiode chip. (b) The CMOS photodiode was fixed on the HP 16058A test fixture which was connected to HP4145 semiconductor parameter analyzer. (c) HP4145 was used to collect and analyze the chemiluminescence generated current. Parts (a) and (b) were placed in a dark box.

For the coupled enzyme assay, H_2O_2 was omitted. Each data point was the average of three measurements. Luminescence of each measurement was obtained from the average of first 10 s in each reaction.

F. Glucose Oxidase Coupled HRP-luminol-H_2O_2 System

The standard condition for this coupled enzyme system included 150 units GOD, 0.64 units HRP, 1 mM luminol, and 0.1 M Tris-HCl buffer (pH 8.6) in a final volume of 1 ml at room temperature. To start the measurement, the mixture of luminol, Tris-HCl buffer, glucose, and GOD that had been incubated for 10 min at room temperature was mixed with HRP. Each data

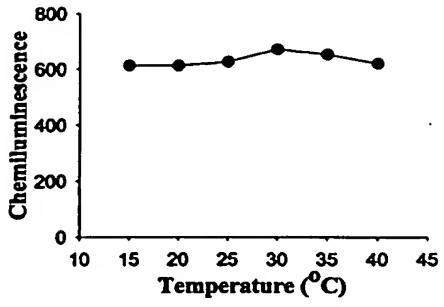


Fig. 3. Effect of temperature on HRP-luminol-H_2O_2 system. The mixture of luminol (1.5 mM,), H_2O_2 (3 mM), and Tris-HCl (100 mM) at pH 8.6 was added into HRP (0.008 unit) to start reaction. All reagents were incubated separately at desired temperature 5 min prior to reaction. Each data point was the average of three measurements.

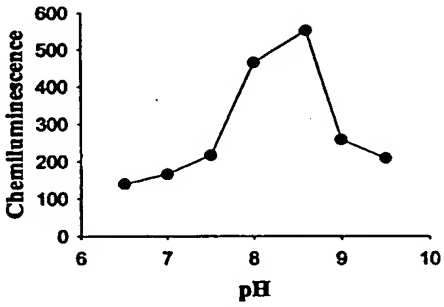


Fig. 4. pH profile of HRP-luminol-H_2O_2 system. The reaction condition was the same as described in Section II for HRP-luminol-H_2O_2 system except that 0.1 M Bis-tris propane buffer (pH 6.5, 7.0, 7.5, 8.0, 8.6, 9.0, or 9.5) was used to replace Tris-HCl. Each data point was the average of three measurements.

point was the average of three measurements. Luminescence of each measurement was obtained from the average of first three seconds in each reaction.

III. Results and Discussion

A. Biochemical Design

The HRP-luminol-H_2O_2 reaction system emits flash type chemiluminescence. The brightest chemiluminescence occurs at the initial steady state of the reaction, and may decay quickly. Reproducible measurements can be obtained according to the procedure described in Section II. The HRP-luminol-H_2O_2 system was impervious to temperature changes in the range of 15 ~ 40 °C as shown in Fig. 3. However, changes in pH significantly affected the activity of HRP-luminol-H_2O_2 system (Fig. 4). According to the results shown in Figs. 3 and 4, pH 8.6 and T = 25 °C were chosen as the standard condition for HRP-luminol-H_2O_2 system. To use the same CMOS platform with enzymes from different sources or in a coupled enzyme system, the reaction condition can be easily modified with the established procedures.

B. Control Data With PMT Measurement System

In addition to obtain control data, the PMT measurement system served as a tool to design the suitable reaction parameters for the novel CMOS detection system. For this purpose,

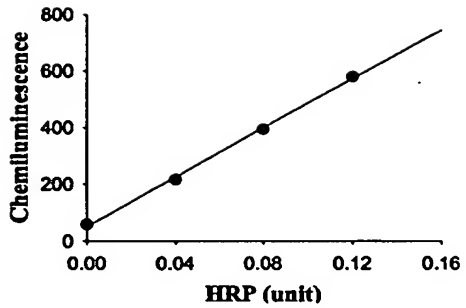


Fig. 5. Control of the HRP in an HRP-luminol-H_2O_2 system. The reaction mixture included H_2O_2 (2.7 mM), luminol (1.24 mM), Tris-HCl buffer (0.1 mM, pH 8.6), and 0~0.12 unit HRP at room temperature. Each data point was the average of three measurements quantified by a fluorometer as described in Section II.

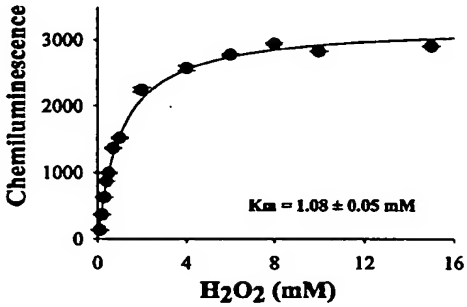


Fig. 6. Control of the variation of H_2O_2 concentration in an HRP-luminol-H_2O_2 system. Each data point was the average of three measurements determined by a fluorometer as described in Section II in a standard condition for HRP-luminol-H_2O_2 system except that 0.2 unit of HRP and 0.1 to 15 mM H_2O_2 were used.

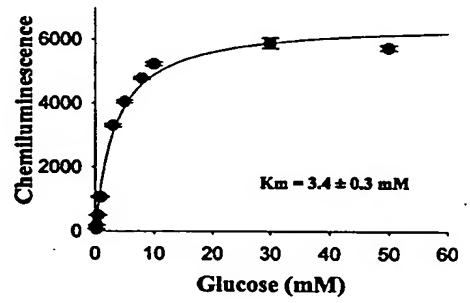


Fig. 7. Control of the variation of glucose concentration in a GOD-coupled HRP-luminol-H_2O_2 system. The glucose concentrations were varied from 0.2 to 50 mM. Each data point was the average of three measurements determined by a fluorometer as described in Section II.

the parameters of the PMT measurement system were adjusted so that comparable data can be obtained from both instrumentation in similar reaction conditions as described in Section II. The enzymatic reactions designed for PMT measurement system (by Hitachi F4500) as shown in Figs. 5–7 could then be compared with those obtained with CMOS/HP4145 detection system shown in Figs. 8–10.

To obtain a useful enzyme assay, a linear relationship between the amount of enzyme used and the enzymatic signal detected must be established. Data shown in Fig. 5 demonstrated

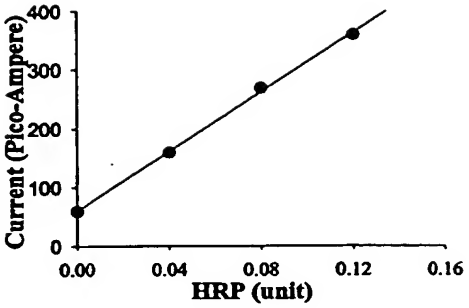


Fig. 8. Enzyme activities observed by CMOS photodiode. The reaction condition was the same as that used by the control detection system described in Fig. 5. Each data point was the average of three measurements.

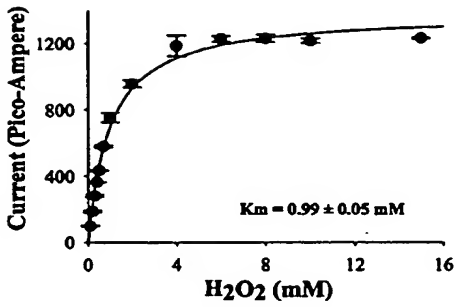


Fig. 9. Effect of H_2O_2 concentration on HRP-luminol-H_2O_2 system observed by CMOS photodiode. The reaction condition was the same as that used by the control detection system described in Fig. 6. Each data point was the average of three measurements.

that chemiluminescence corresponded linearly to enzyme activity, so that the amount of light observed was a direct measurement for the quantity of active enzyme present. Fig. 6 gave the relationship of the substrate concentration, $[H_2O_2]$, and the HRP-luminol-H_2O_2 system. K_m is a constant that describes this relationship and is defined by Michaelis–Menten equation, $v = V_{max}[H_2O_2]/(K_m + [H_2O_2])$. V_{max} is the reaction rate measured at saturation of $[H_2O_2]$ and v is the reaction rate at each $[H_2O_2]$. Typical reaction profiles were obtained with the variation of substrates, H_2O_2 or glucose, as shown in Figs. 6 or 7, respectively. Fig. 7 describes the relationship of the glucose concentration and the coupled enzyme system. The concentration of H_2O_2 and glucose in an unknown sample can be determined according to Figs. 6 and 7, respectively.

C. Enzymatic Data Obtained From CMOS/4145 Detection System

The rate of the enzymatic reaction obtained with CMOS/4145 detection system was described by the current in the range of picoampere to nanoampere (Figs. 8–10). Similar to that observed by the control PMT measurement system, the unit of the reaction rate measured should be considered as an arbitrary unit. The enzymatic signal can be magnified electronically and is dependent on the sensitivity of the instrument used. A linear relationship between the amount of HRP used and the current generated through the CMOS photodiode was obtained as shown in Fig. 8. A dark current at about 56 pA was also observed and was considered as a background that was substrated for the following reactions measured with CMOS photodiode. A typical reaction profile of HRP-luminol-H_2O_2 system with the variation of $[H_2O_2]$ was obtained and shown in Fig. 9. The K_m of H_2O_2 determined with the CMOS photodiode was 1 mM. In a coupled enzyme system, monitored by CMOS/4145 detection system also gave a typical reaction profile. The K_m of glucose was about 5 mM in the GOD coupled HRP-luminol-H_2O_2 system. The results shown in Figs. 8–10 all indicated that useful enzymatic data were obtained with CMOS/4145 detection system.

D. Comparison of Enzymatic Data Obtained From PMT and CMOS Photodiode

The results shown in Figs. 8–10 were in excellent agreement with those shown in Figs. 5 and 7. Linear response of the

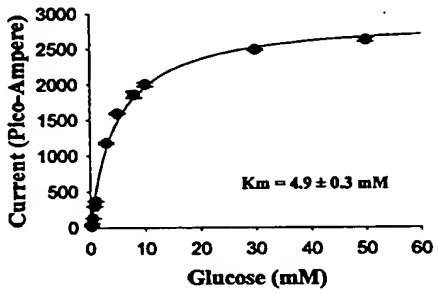


Fig. 10. Glucose concentration curve observed by CMOS photodiode. The reaction condition of the coupled enzyme system was the same as that used by control detection system described in Fig. 7. Each data point was the average of three measurements.

enzyme used and signal observed by CMOS/4145 detection system (Fig. 8) was similar to that of PMT system (Fig. 5) and both gave satisfactory results. Differences in Kms obtained in both detection systems were in an acceptable range (Figs. 6 and 7 versus Figs. 9 and 10, respectively). Figs. 7 and 10 show that the activity of HRP-luminol-H_2O_2 system was dependent on the concentration of glucose. The K_m of glucose was determined to be 3.4 ± 0.3 mM and 4.9 ± 0.3 mM with PMT instrument and CMOS photodiode, respectively. The concentration of glucose in biological samples (e.g., blood) is in the mM range. These results demonstrate that concentration of glucose can be determined with a GOD coupled HRP-luminol-H_2O_2 system by a CMOS photodiode.

IV. Conclusion

1) We demonstrated that a CMOS photodiode manufactured by a standard CMOS process is useful for biochemical measurements. This result and the characteristics of CMOS (low price, small size, low power consumption, and short response time) may make CMOS photodiodes a very attractive sensor for clinical diagnostics.
2) We used glucose and GOD to show that CMOS photodiode and HRP-luminol-H_2O_2 system can be used as a platform for coupled enzyme assay to identify and quantify a variety of biochemicals (listed in Table I).

3) The sensitivity of this CMOS chip can be improved further to become a routine biochemical and enzyme sensor. PMT instrument is still a much more sensitive sensor (one hundred fold or more) than that of the CMOS diode. The preparation of a more responsive CMOS diode for biochemical sensing is now in progress.

ACKNOWLEDGMENT

The authors wish to thank the reviewers for their suggestions.

REFERENCES

[1] Y.-S. Yang, U. Lu, and B. C. P Hu, "Prescription chips: toward the development of enzyme and biochemical Cmos chips," *IEEE Circuits Devices Mag.*, vol. 18, pp. 8–16, 2002.
[2] S. M. Sze, *Semiconductor Sensors*. New York: Wiley, 1994.
[3] M. A. Deluca, *Method in Enzymology*. New York: Academic, 1978.
[4] S. M. Sze, *Physics of Semiconductor Devices*, 2nd ed. New York: Wiley, 1981.
[5] E. K. Bolton, G. S. Sayler, D. E. Nivens, J. M. Rochelle, S. Ripp, and M. L. Simpson, "Integrated CMOS photodetectors and signal processing for very low-level chemical sensing with the bioluminescent bioreporter integrated circuit," *Sens. Actuators B*, vol. 85, pp. 179–185, 2002.
[6] L. J. Kricka, J. C. Voyta, and I. Bronstein, *Method in Enzymology*. New York: Academic, 2000.

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摘要：

我們揭露了一種創新的生化感測方法及其具潛力性的新穎應用方式，其係透過一個利用標準 0.5 微米製程所製造的複合式金氧半導體（CMOS）感光晶片以量測生化反應所產生之光，此光係利用山葵根過氧化酵素（HRP）催化酵素反應形成，可經由 CMOS 感光晶片及一標準半導體參數分析儀（HP4145）加以感測分析，並可設計作爲偵測過氧化氫（H_2O_2）濃度之平台反應，此酵素反應之動態會再經由一標準且精密的螢光度量計（Hitachi F-4500）來加以測定比較，而上述二儀器所測得之結果十分相似。以葡萄糖氧化酵素爲例，我們進一步地證明了利用該 CMOS 系統之 HRP 反應平台可以測定其他 H_2O_2 所產生之反應，而此結論指出 CMOS 晶片於生物量測及各種健康因素診斷時能發揮重大應用。

生化設計：

該 HRP-螢光- H_2O_2 之反應系統會放射出閃爍類型的化學發光，最明亮的化學發光發生於反應之初的穩態，且可能會迅速衰減，而可重覆實施的量測方法可根據第二節所述之程序以獲得，另，如第三圖所示，此 HRP-螢光- H_2O_2 反應系統於溫度範圍 15～40℃內並不受溫度變化之影響，然而，pH 值的變化會大幅影響此 HRP-螢光- H_2O_2 反應系統之活性（如第四圖所示），而根據第三圖及第四圖所示之結果，pH 值 8.6 及溫度 25℃被選爲此 HRP-螢光- H_2O_2 反應系統之標準條件，而若欲使用同樣的 CMOS 平台搭配不同酵素來源或某一耦合的酵素系統，則亦可輕易地藉由已建立之程序來調整反應條件。

CMOS/4145 偵測系統所得之酵素數據：

CMOS/4145 偵測系統係以微微安培至毫微安培來表示酵素反應速率（參照第八圖至第十圖），這與光電倍增管量測系統（PMT）的顯示相似，且反應速率的單位可視爲一任意單位，而酵素反應的訊號可以電子方式進行放大且與所用儀器之靈敏度息息相關。第八圖顯示出 HRP 之份量與 CMOS 感光二極體所測得之電流間的線性關係，由圖可觀察到一大小爲 56 微微安培之暗電流，其被視爲此 CMOS 感光二極體之量測基準，第九圖則顯示出一隨著 H_2O_2 濃度改變之 HRP-螢光-H_2O_2 系統的標準反應曲線，此 CMOS 感光二極體所測定的 H_2O_2 之 K_m 值爲 1mM，而在一耦合的酵素系統中，利用 CMOS/4145 偵測系統亦可偵測得到一標準反應曲線，另於 GOD 耦合 HRP-螢光-H_2O_2 之系統中，葡萄糖的 K_m 值大約是 5mM。第八圖至第十圖所呈現之結果均指出 CMOS/4145 偵測系統可測得有用之酵素數據。

結論：

（1） 我們證明了利用標準 CMOS 製程所製造之 CMOS 感光二極體非常適用於生化感測，此結果以及 CMOS 的特性（低成本、體積小、低消耗功率以及很短的反應時間）都可能使 CMOS 感光二極體成爲令人感興趣的臨床

檢測感測器。

（2） 我們使用了葡萄糖及葡萄糖過氧化酵素以展現 CMOS 感光二極體及 HRP-螢光-H_2O_2 系統可被用做酵素檢測的平台，並能藉其對不同生化試劑作定性及定量分析（如表一所示）。

（3） 此 CMOS 晶片之靈敏度可進一步地改善以作爲標準的生化及酵素感測器，PMT 儀器仍然是一種較 CMOS 二極體靈敏得多的感測器（靈敏一百倍以上），然而更加靈敏的生化感測 CMOS 二極體目前已在準備研發中。